# Improved prediction of meat and bone meal metabolizable energy content for ducks through in vitro methods[1]

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**ABSTRACT** Apparent metabolizable energy (AME) of meat and bone meal (MBM) for poultry is highly variable, but impractical to measure routinely. Previous efforts at developing an in vitro method for predicting AME have had limited success. The present study uses data from a previous publication on the AME of 12 MBM samples, determined using 288 White Pekin ducks, as well as composition data on these samples. Here, we investigate the hypothesis that 2 noncompositional attributes of MBM, particle size and protease resistance, will have utility in improving predictions of AME based on in vitro measurements. Using the same MBM samples as the previous study, 2 measurements of particle size were recorded and protease resistance was determined using a modified pepsin digestibility assay. Analysis of the results using a stepwise construction of multiple linear regression models revealed that the measurements of particle size were useful in building models for AME, but the measure of protease resistance was not. Relatively simple (4-term) and complex (7-term) models for both AME and nitrogen-corrected AME were constructed, with R-squared values ranging from 0.959 to 0.996. The rather minor analytical effort required to conduct the measurements involved is discussed. Although the generality of the results are limited by the number of samples involved and the species used, they suggest that AME for poultry can be accurately predicted through simple and inexpensive in vitro methods.

**Key words:** duck, meat and bone meal, metabolizable energy, particle size, protein digestibility

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## INTRODUCTION

In poultry diets, the greatest portion of overall expense goes to meeting the requirement for dietary energy (De Lange and Birkett, 2005). To avoid losses due to supplying either too much or too little energy, the ME content of each ingredient is required. Past reports have shown that the AME content of meat and bone meal (**MBM**) for poultry varies over a wide range (Dolz and de Blas, 1992; Adedokun and Adeola, 2005). Because direct measurement of AME requires expensive and time-consuming in vivo studies, routine determination of AME is impractical. A rapid, inexpensive in vitro method for estimating AME of MBM for poultry would allow this feedstuff to be used more effectively.

Researchers have repeatedly attempted to use proximate composition of MBM (and poultry by-product meal, a closely related substance) to generate reliable estimates of ME (Dolz and de Blas, 1992; Dale et al., 1993; Adedokun and Adeola, 2005; Ahmadi et al., 2008; Perai et al., 2010). In general, these efforts have met with limited success unless they resorted to complex techniques such as artificial neural networks. Some studies have improved their predictions by supplementing their data with measurements of gross energy (**GE**). Because the equipment used to measure GE is not always available in feed labs (Robbins and Firman, 2006), a method that does not require GE would be more practical.

There is evidence that the variation in AME is at least partly explained by noncompositional factors. Intensity of heat treatment is known to impact digestibility of animal protein meals (Johns et al., 1987; Wang and Parsons, 1998). Particle size of various feedstuffs is often observed to have an effect on AME (Dänicke et al., 1998; Jiménez-Moreno et al., 2010). The present work investigates whether methods readily available to basic feed labs for analysis of particle size and protein


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digestibility can improve prediction of AME in MBM for ducks.

## MATERIALS AND METHODS

Data on AME, $AME_n$, and proximate composition of the 12 MBM samples used in the present study are from an earlier publication of one of the authors (Adedokun and Adeola, 2005); this publication provides detailed Materials and Methods information. Briefly, AME and $AME_n$ were determined by substitution of 0, 5, or 10% MBM into a basal corn-soybean meal diet for 288 White Pekin ducks. The MBM used in the present study are the same samples used in the previous study. Crude protein values used here are the result of new analyses made using the methods described below, but they are in good agreement with the previously reported values (data not shown).

Due to the limited amounts of MBM samples from the previous study, different MBM samples were used in the method development portion of this study. Labeled ME1, ME2, and ME3, these were obtained from John Kuhni Sons Inc. (Nephi, UT), Darling International (Irving, TX), and Hormel Foods (Austin, MN), respectively.

### Protease Resistance and Particle Size

Protease resistance and particle size were determined according to AOAC official method 971.09—Pepsin digestibility of animal protein feeds (AOAC International, 2009), with the following modifications. Fat-extracted MBM samples (10 g) were placed atop a stack comprising a US Standard #20 sieve (850-μm openings), a #35 sieve (500-μm openings), and a pan and shaken in a Ro-Tap (model RX-29, W. S. Tyler, Cleveland, OH) for 10 min. The cumulative proportion of sample retained on each sieve was determined. All 3 size fractions were recombined and treated in a liquid nitrogen-cooled pulverizing mill (model 6800, Spex SamplePrep, Metuchen, NJ). The milling program comprised an initial 10-min chilling period followed by 2 cycles of 2-min milling and 2-min chilling between cycles.

The pepsin-HCl solution was prepared either as specified in the standard or with 1/10th, 1/100th, 1/1000th the specified pepsin concentration, or with no pepsin. Instead of the rotator specified in the standard, we used an orbital shaker (model SWB 5050, Labnet, Woodbridge, NJ); the rotators specified in the standard are no longer manufactured and results of a multilaboratory study (Miller et al., 2002) found that substitution of the rotator with an orbital shaker operating at 140 to 200 orbits/min had an insignificant effect on the assay.

Nitrogen content of the residual material was determined by the Kjeldahl method, but rather than AOAC method 945.01 specified by the standard, the more modern AOAC method 920.39 was substituted. A system comprising a Tecator digestion unit and a Kjeltec 8100 distillation unit (Foss North America, Eden Prairie, MN) was used for the analysis. A nitrogen-to-protein conversion factor of 5.37 was used based on the work of Sriperm et al. (2011). All analyses were conducted in triplicate.

### Statistical Analysis

Analysis was performed using Minitab 14.1 (Minitab Inc., State College, PA). Pearson correlation coefficients between all pairs of first-order experimental predictors were examined to confirm acceptable independence. The stepwise regression routine was used to generate and compare candidate regression equations. The routine was allowed to add or subtract terms from the model one-at-a-time using a significance level of $\alpha = 0.05$. Selection from the population of strongly predictive equations was made according to practical considerations rather than statistical criteria and is discussed in the following section.

## RESULTS AND DISCUSSION

The previous study (Adedokun and Adeola, 2005) dropped results from 2 of the 12 MBM samples used in the study as outliers; in the present study, we have taken a more conservative approach. One of the 2 dropped in the previous study has been retained here. The other, sample #1, is an extreme outlier in many respects (Tables 1 and 2); in almost every characteristic measured, it had either the minimum or maximum value in the group of samples. Compared with surveys of MBM properties (Hendriks et al., 2002; Garcia et al., 2006) and the characteristics normally specified in contracts for MBM (National Renderers Association, 2003), this material does not have the characteristics of MBM normally offered to feed ingredient producers, and so we feel justified in eliminating it from the data set used in the subsequent analysis.

The hypothesis behind this work is that particle size and protease resistance may both account for some of the variation in AME of MBM. Two experimental methods were developed to measure these properties, using equipment and techniques appropriate for standard feed labs. Protease resistance was measured using a modified version of the pepsin digestibility assay, a somewhat discredited method for in vitro measurement of protein digestibility. To avoid confounding between measurements of protease resistance and particle size, steps were taken to minimize particle size differences between samples and particle size distribution within samples, before testing for protease resistance. Milling the samples in a cryogenic mill effectively reduced the within-sample particle size distribution by breaking the largest particles while leaving the smallest mostly intact (Figure 1A). Noting that the results are presented on a logarithmic scale, the range of particle sizes present after milling is much narrower. Milling also reduced the differences in geometric mean particle diameter between samples (Figure 1B). Milling more than

**Table 1.** Energy and nutrient composition of meat and bone meal (MBM) samples on a DM basis[1]

| MBM sample | AME, kcal/kg DM | $AME_n$, kcal/kg DM | CP,[2] g/kg | CF,[2] g/kg | Moisture, g/kg | P, g/kg | Ca, g/kg |
|---|---|---|---|---|---|---|---|
| 1 | 3,533 | 3,280 | 446.5 | 91.1 | 78.8 | 61.7 | 145.8 |
| 2 | 2,106 | 2,066 | 476.5 | 97.7 | 36.3 | 46.5 | 106.4 |
| 3 | 3,394 | 2,884 | 538.6 | 110.8 | 54.9 | 28.3 | 61.6 |
| 4 | 2,349 | 2,279 | 524.0 | 140.8 | 60.1 | 25.6 | 54.3 |
| 5 | 1,781 | 1,772 | 509.2 | 110.3 | 37.7 | 40.8 | 93.5 |
| 6 | 3,578 | 3,256 | 579.9 | 96.9 | 17.9 | 26.7 | 61.6 |
| 7 | 2,421 | 2,534 | 508.0 | 93.3 | 20.7 | 43.4 | 102.5 |
| 8 | 2,934 | 2,927 | 514.6 | 115.5 | 9.3 | 39.4 | 88.0 |
| 9 | 3,916 | 3,662 | 519.3 | 106.5 | 10.6 | 36.1 | 84.3 |
| 10 | 3,153 | 2,916 | 488.0 | 120.5 | 28.1 | 36.8 | 85.1 |
| 11 | 3,434 | 3,213 | 572.8 | 113.4 | 26.6 | 27.4 | 66.3 |
| 12 | 3,080 | 2,965 | 520.5 | 151.2 | 30.8 | 37.6 | 87.2 |
| Minimum | 1,781 | 1,772 | 446.5 | 91.1 | 9.3 | 25.6 | 54.3 |
| Maximum | 3,916 | 3,662 | 579.9 | 151.2 | 78.8 | 61.7 | 145.8 |

[1]From Adedokun and Adeola (2005), except for CP, which are original data.
[2]CP = N × 5.37; CF = crude fat.

one cycle had little additional effect on either overall mean particle diameter of a sample or differences in mean particle diameter between samples. Particle size did not, however, affect measurements of protease resistance (Figure 1C). Some milling is still required to ensure that the small subsamples of MBM used in the assay are representative, so samples were milled for 2 cycles in subsequent experiments.

Attempting to adapt the pepsin digestibility method for fishmeal analysis, earlier investigators (Olley and Pirie, 1966; Amato and Griffiths, 1986; Miller et al., 2002) observed that use of lower pepsin concentrations resulted in a more sensitive assay; poultry nutritionists have also observed this improved sensitivity (Johnston and Coon, 1979; Parsons et al., 1997). Using 3 different samples of MBM, we observed a similar increase in assay sensitivity at reduced pepsin concentrations (Figure 2). At the standard pepsin concentration of 2 g/L, the proportion of undigested protein in all samples was in the range of 7.1 to 18.0%, with ME1 and ME3 producing very similar values. With each progressive 10-fold dilution of the pepsin concentration, the results from the 3 samples spread over a wider range, resulting in a range of 24.8 to 63.0% undigested protein at the lowest pepsin concentration tested. At this low concentration, ME1 and ME3 are clearly differentiated. In subsequent experiments, protease resistance was measured using 0.002 g/L of pepsin (Table 2).

Particle size analysis is a straightforward and commonplace analysis applied to feed ingredients. In the present study we used a simplified method for particle size analysis which would allow greater sample throughput in a feed laboratory, compared with standard methods. The percentage of an MBM sample retained on just 2 different, relatively large-opening sieves was determined (Table 2). If half-height sieves are used, a standard sieve shaker can process 5 samples in parallel, in one 10-min run.

In the previous report using these MBM samples (Adedokun and Adeola, 2005), regression equations with 6 predictors each were generated for AME and $AME_n$, giving $R^2$ values of 0.552 and 0.598, respectively. These included 2 predictors, GE and ash, which are not routinely determined by renderers or their customers [David Kirstein, Darling International, personal communication; "(Ashing values may be available) only

**Table 2.** Experimental predictors of meat and bone meal (MBM) metabolizable energy

| MBM sample | CP undigestible, % | Mass retained on #35 sieve, % | Mass retained on #20 sieve, % |
|---|---|---|---|
| 1 | 21.17 | 2.71 | 0.56 |
| 2 | 43.75 | 13.27 | 1.23 |
| 3 | 27.80 | 15.05 | 2.26 |
| 4 | 33.19 | 26.05 | 1.88 |
| 5 | 28.51 | 31.85 | 3.94 |
| 6 | 44.14 | 30.10 | 3.31 |
| 7 | 31.63 | 19.35 | 1.10 |
| 8 | 38.21 | 26.20 | 6.03 |
| 9 | 33.79 | 10.07 | 1.69 |
| 10 | 31.81 | 16.32 | 0.69 |
| 11 | 43.24 | 20.01 | 0.68 |
| 12 | 36.40 | 23.52 | 10.56 |
| Minimum | 21.17 | 2.71 | 0.56 |
| Maximum | 44.14 | 31.85 | 10.56 |

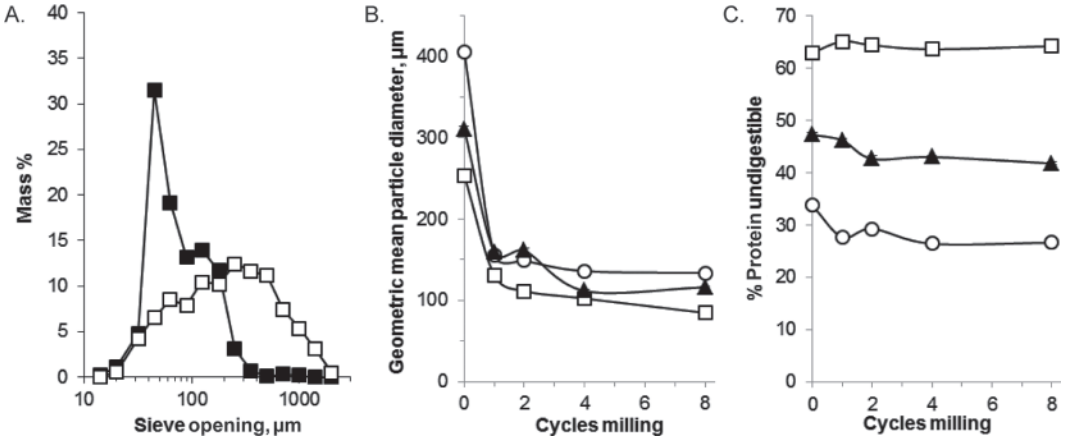


**Figure 1.** A) Particle size distribution of meat bone meal sample ME2 before (□) and after (■) 8 milling cycles. B) Geometric mean particle diameter over a range of milling durations. C) Percentage of undigestible protein over a range of milling durations. The experimental material in B and C are meat bone meal samples ME1(○), ME2(□), and ME3(▲). Error bars representing one standard deviation are present for all data points but are obscured by datapoint markers in many cases.

because (the samples) need to be ashed to run the other mineral analyses"]. The present study does not include either GE or ash in the regression modeling.

The proximate composition, size, and protease resistance data on 11 samples used in the earlier study (Adedokun and Adeola, 2005) were used to build regression models for predicting AME and $AME_n$. Examination of the Pearson correlation coefficients between the predictors (Table 3) showed that most pairs of predictors were insignificantly dependent upon one another. The few cases of dependence had clear bases in the makeup of MBM (e.g., Ca and P are highly correlated because of the fixed composition of bone). No single predictor was significantly correlated with either AME or $AME_n$. The (statistically insignificant) correlation coefficients between AME or $AME_n$ and the predictors were generally of the sign that would be expected—a positive correlation with crude protein because of the contribution of protein to energy, negative correlations with calcium, phosphorus, and moisture due to their noncaloric nature and displacement of caloric components. There was a negative coefficient for mass retained on a #35 sieve, consistent with the hypothesis that large particles are less digestible and depress AME and $AME_n$. Crude fat and mass retained on a #20 sieve had near zero correlation coefficients, perhaps due to the small dynamic range of these parameters in the data set. The percentage of undigested crude protein had an unexpected positive correlation coefficient with AME and $AME_n$, but as will be shown later, this parameter was not a useful predictor.

The regression modeling included cross products between predictors in cases where the cross product seemed potentially meaningful and predictive. For example, crude protein × % undigested protein conceptually has information about the concentration of protein available to contribute to ME. Cross products such as crude fat × % undigested protein were not included because they were not clearly meaningful. The full list of cross products investigated is shown in Table 4.

One approach to building useful regression models in this case is to consider the analytical values that are routinely determined for lots of MBM to be free, in the sense that no extra analytical effort is required to obtain them. With this approach, the free predictors (crude protein, crude fat, calcium, phosphorus, and moisture) are included in every model. Stepwise regres-

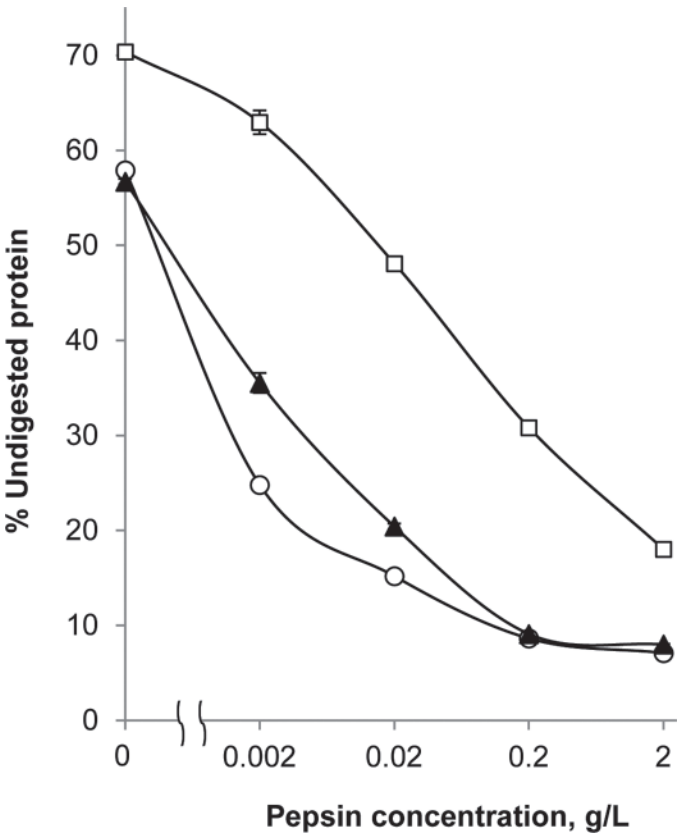


**Figure 2.** Proportion of 3 meat bone meal samples (○ ME1, □ ME2, ▲ ME3) remaining undigested using a range of pepsin concentrations. Error bars representing one standard deviation are present for all data points but are obscured by data point markers in many cases.

**Table 3.** Pearson correlation coefficients between analytical characteristics of 11 meat and bone meal samples[1,2]

| | AME | $AME_n$ | CP | CF | Moisture | P | Ca | CP undigestible | #35 sieve |
|---|---|---|---|---|---|---|---|---|---|
| $AME_n$ | 0.98* | | | | | | | | |
| CP | 0.56 | 0.52 | | | | | | | |
| CF | 0.00 | 0.00 | −0.04 | | | | | | |
| Moisture | −0.40 | −0.55 | −0.08 | 0.38 | | | | | |
| P | −0.52 | −0.42 | −0.80* | −0.28 | −0.34 | | | | |
| Ca | −0.46 | −0.35 | −0.74* | −0.31 | −0.40 | 0.99* | | | |
| CP undigestible | 0.20 | 0.26 | 0.34 | −0.18 | −0.38 | −0.07 | −0.03 | | |
| #35 sieve | −0.36 | −0.34 | 0.35 | 0.21 | 0.05 | −0.21 | −0.23 | −0.06 | |
| #20 sieve | 0.00 | 0.05 | 0.03 | 0.59 | −0.13 | 0.13 | 0.10 | −0.03 | 0.43 |

[1]Sample number 1 excluded from the analysis.

[2]CP = N × 5.37; CF = crude fat; #35 (#20) sieve = % mass retained on a #35 (#20) sieve.

*$P < 0.05$.

sion modeling, with the constraint that these predictors are always retained, produced highly predictive 7-parameter regression models for both AME and $AME_n$ (Table 5, equations 1 and 2). In both cases, the modeling routine selected the masses retained on #20 and #35 sieves as significant contributors to the fit of the equations. Neither percent of undigested protein nor any of the cross products were selected.

Construction of a 7-parameter model based on 11 data points may result in an overfit model. An alternative approach for regression model building is to prefer more parsimonious models even if this means ignoring free data. To implement this approach, the stepwise regression routine was set up to use a model with all of the free predictors as a starting point but was allowed to drop these predictors one-at-a-time if their contribution to the fit of the model did not meet the significance criterion. This resulted in 4-parameter models for AME and $AME_n$, with $R^2$ values of 0.959 and 0.988, respectively (Table 5, equations 3 and 4). For both AME and $AME_n$, neither crude fat nor calcium is used as a predictor. Crude protein is included only in cross products with MBM particle size measurements. That some composition parameters can be eliminated without losing much predictive power is presumably related to the high degree of redundancy between the various composition parameters; MBM with relatively high protein concentration always has relatively low calcium and phosphorus concentrations, for example.

Both modeling approaches highlight the utility of particle size data in predicting AME and $AME_n$. Compared with regression models fit to all 5 free predictors (Table 5, equations 5 and 6), models fit to all 5 free predictors and particle size terms (Table 5, equations 1 and 2) fit the data much better. Similarly, parsimonious models (Table 5, equations 3 and 4) lose much of their predictive power if the particle size terms are removed (Table 5, equations 7 and 8). Measurements of particle size seem to capture some information about ME that is not to be found in the composition measurements.

The particles retained on both #20 and #35 sieves are in the upper end of the size range typical for MBM (Garcia et al., 2006). These results do not, however, provide an obvious route to improving the actual AME of MBM for ducks. In the 7-parameter models (Table 5) the coefficients on 'mass retained on a #20 sieve' and 'on a #35 sieve' have opposite signs, making it difficult to interpret the effect of particle size on energy availability. It may be that the AME of MBM can be elevated through finer grinding, but this present work was not designed to examine that question directly.

The measure of protease resistance we employed in this work did not have much predictive value, but this does not necessarily discredit the utility of a differently designed measure of protease resistance. Although predictors used in the this study accounted for the variation of AME and $AME_n$ very well, they did not account for the difference between GE and AME in this population of samples; that is, they do not explain the reasons that ducks can only access a limited portion of the total energy available in MBM. The stepwise regression routine was used again with the same settings used when generating the parsimonious regression equations above, to see if the data could predict the difference between GE and AME. The routine dropped all of the parameters, one-by-one, resulting in no equation. Because protein provides the largest proportion of GE in MBM and the digestibility of MBM protein is known to vary along with the processing conditions used to produce it (Wang and Parsons, 1998), it is reasonable

**Table 4.** Cross products of predictors considered for regression model building

| Predictor | Abbreviation |
|---|---|
| CP × % protein undigestible | CP × CP undigestible |
| CP × % mass retained on #20 sieve | CP × #20 |
| CP × % mass retained on #35 sieve | CP × #35 |
| CP × % protein undigestible × % mass retained on #20 sieve | CP × CP undigestible × #20 |
| CP × % protein undigestible × % mass retained on #35 sieve | CP × CP undigestible × #35 |

**Table 5.** Multiple linear regression equations for the prediction of AME and $AME_n$ using in vitro measurements[1]

| Equation | AME | Const. | CP | CF | Moisture | P | Ca | #20 sieve | #35 sieve | CP × #20 | CP × #35 | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | AME | 23,299 | −191.9 | −24.6 | −21.5 | −383.9 | 89.7 | 200.8 | −56.4 | | | 0.991 |
| 2 | $AME_n$ | 13,464 | −87.7 | −8.8 | −24.4 | −210.4 | 45.4 | 109.2 | −44.5 | | | 0.996 |
| 3 | AME | 7,729 | | | −25.8 | −83.2 | | | −62.0 | 139.0 | | 0.959 |
| 4 | $AME_n$ | 6,919 | | | −27.3 | −71.2 | | | | 114.3 | −89.0 | 0.988 |
| 5 | AME | 12,792 | −96.7 | −0.5 | −29.9 | −211.9 | 44.9 | | | | | 0.685 |
| 6 | $AME_n$ | 10,433 | −77.6 | 2.2 | −29.4 | −179.4 | 41.6 | | | | | 0.762 |
| 7 | AME | 6,154 | | | −26.8 | −68.5 | | | | | | 0.645 |
| 8 | $AME_n$ | 5,422 | | | −26.5 | −52.4 | | | | | | 0.710 |

[1]CP = N × 5.37; g/kg; CF = crude fat (g/kg); #20 (#35) sieve = % mass retained on a #20 (#35) sieve; CP × #20 (#35) = CP × % mass retained on #20 (#35) sieve.

to suspect that a properly designed measure of protease resistance would explain a portion of this variation. Such a measure could, in turn, assist renderers in modifying processing conditions to more fully reach the energetic potential of MBM for ducks.

In conclusion, it appears that proximate composition along with particle size data can be used to produce accurate predictions of AME and $AME_n$ for ducks. Further studies with larger numbers of samples and other poultry species could produce a validated method that would provide feed formulators with routine access to more useful data on MBM energy. Such availability would allow MBM to be used in poultry diets with greater confidence that its contribution to total dietary energy is well understood.

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